United States Department of Agriculture
Agricultural Marketing Service
Science & Technology
Monitoring Programs Office
8609 Sudley Rd., Ste. 206
Manassas, VA 20110

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TO: See Distribution List

FROM: Martha Lamont, Director
Monitoring Programs Office

SUBJECT: Microbiological Data Program Plan, January through June 2006

This Program Plan serves as the current Statement of Work for the period January 1, 2006, through June 30, 2006, for each State participating in the Microbiological Data Program (MDP). This document also stipulates work assignments for the Federal facility participating in MDP.

I. ADMINISTRATIVE UPDATES

A. Personnel: Program participants are reminded to keep MDP management informed of any critical equipment purchases, staffing issues, or expected increases in rent or sample turn-around-time (e.g., due to laboratory or office renovation/relocation). This information is required under the terms of MDP Cooperative Agreements (Section II, Responsibilities) between USDA and participating States.

B. Summary Status: The 2004 MDP Data Summary is being drafted by Monitoring Programs Office (MPO) staff.

C. MPO Personnel: Dr. Jane Tang has been hired as a Microbiologist and joined MDP on October 17, 2005. Lisa Pearmund has assumed the Sampling Coordinator position and Sharon Hane, Management Analyst, has assumed responsibility for the cooperative agreements and reimbursements.

D. Financial/Cooperative Agreements: Cooperative agreements will be issued when the FY2006 Budget is signed by the President.

E. MDP Program Meetings: On September 27-29, 2005, MPO held a joint Federal/State Meeting for MDP and the Pesticide Data Program (PDP) in Denver, Colorado. Administrative, sampling, and technical issues for MDP and PDP were covered. A Quality Assurance (QA)/Technical Meeting is planned for March 27-31, 2006, at the

Virginia Division of Consolidated Laboratory Services (DCLS), Richmond, Virginia. QA Officers and technical staff from each laboratory are invited to attend.

F. Electronic Transfer of Data:

RDE Version Upgrades: The current version of the Web-based RDE system was installed in June 2005 and the next version upgrade is tentatively scheduled for March 2006. A version upgrade for the RDE e-SIF system for laptops/palmtops is in development to add selection lists for populating the post-harvest treatment field and to allow the user to change an existing Sample ID that has an erroneous entry. The e-SIF upgrade is expected to be available in December 2005. MPO maintains a Change Request Database to capture all problems identified and suggestions made regarding the RDE system.

RDE Web Addresses: RDE users in the laboratories should be using the Secure Socket Layer (SSL) site address to access the Web-based RDE system on the AMS production Web server. The SSL address is https://www.ams.usda.gov/rde. The only difference is the addition of the letter "s" following "http". This SSL technology is used to encrypt all data passed between the user's computer and the central web server. If the secure site is not available, the AMS developmental Web server with address http://151.121.3.127/RDE can be used.

II. PROGRAM SAMPLING AND TESTING UPDATES

A. Sampling: Shipping Charts are distributed quarterly to Sampling Managers by MPO. Cantaloupe, tomatoes, green onions, and lettuce (leaf or romaine) will continue. Effective January 1, 2006, alfalfa sprouts will be collected in all States in place of cilantro/parsley; Texas will continue the sampling of alfalfa sprouts, which began as a pilot program on October 1, 2005. Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will continue to be shipped to the AMS National Science Laboratory [NSL (US4)]. All other samples will be sent to the laboratory for that collection State.

B. Testing: Cantaloupe, tomatoes, green onions, and lettuce (leaf or romaine) will continue. Effective January 1, 2006, alfalfa sprouts will be tested in all States and cilantro/parsley will be discontinued. Samples collected in Maryland will be tested by the Ohio laboratory (OH4) and those collected in Texas will continue to be tested by NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

New Wash Methods:

(1) The current wash solution (Buffered Peptone Water with 0.1% Tween) will be replaced with Universal Preenrichment Broth (UPB), which will also serve as the single preenrichment broth for all target organisms. The change to UPB as a single wash and preenrichment broth for culturing different bacteria will streamline MDP processes. The new procedure was developed following successful feasibility and validation studies, performed by DCLS.

(2) The sample preparation procedure will include a stomaching method for alfalfa sprouts. The determination of optimum stomaching was performed by DCLS.

(3) For all commodities, a DNA extraction step will be performed on bacterial cultures enriched from produce washes followed by testing for the target organism by BAX polymerase chain reaction (PCR) and multiplex PCR (mPCR) where applicable.

Target Microorganisms:

(1) *Escherichia coli* (*E. coli*): MDP laboratories will continue testing all samples for *E. coli* using ColiComplete®. Method procedures are detailed in Standard Operating Procedure (SOP) MDP-MTH-01, *Escherichia coli* MPN Method.

(2) MDP laboratories will screen samples that were positive for *E. coli* (by SOP MDP-MTH-01) for pathogenic *E. coli* according to SOP MDP-MTH-07, Detection of Pathogenic *E. coli* in Fresh Produce by Multiplex PCR (mPCR) and Cultural Isolation and Identification. The mPCR assay tests for two types of pathogenic *E. coli*: (a) shiga toxin-producing *E. coli* (STEC) which contain genes coding for shiga toxins (Stx-1 and Stx-2); and (b) enterotoxigenic *E. coli* (ETEC) containing genes coding for the heat labile (LT-1) and heat stable (ST-1) toxins. Cultural procedures for the isolation of pathogenic *E. coli* are included in SOP MDP-MTH-07.

(3) MDP laboratories will continue to screen all samples for *Salmonella* (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-04, Detection of *Salmonella* in Fresh Produce by BAX® PCR. Presumptive positive samples are subjected to enrichment and isolation as described in SOP MDP-MTH-03A, Isolation and Identification of *Salmonella* from Fresh Produce Using Cultural Methods.

(4) MDP laboratories will continue to screen all samples for *E. coli* O157:H7 (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX® PCR. Presumptive positive samples are subjected to immunomagnetic separation (IMS) procedures and cultural confirmation, as described in SOP MDP-MTH-06, *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Identification.

C. Quality Assurance:

Proficiency Testing Program: The next proficiency test, which will include a non-toxigenic *E. coli* O157:H7 strain, is currently under development.

Method Validations: The UPB/BAX® Method Validation for *Salmonella* and *E. coli* O157:H7 was performed by DCLS. The DNA extraction and alfalfa sprouts method validations were performed by DCLS. Ohio is continuing to explore the feasibility of the Fluorocult® method for *E. coli* screening.

Method Verifications: A method verification combining UPB, ColiComplete®, and DNA extraction for all commodities, including alfalfa sprouts, must be successfully completed by each laboratory prior to implementation of revised SOPs on January 1, 2006.

SOPs: SOPs are posted to the MDP website when distributed to program participants. http://www.ams.usda.gov/science/MPO/SOPs.htm.

The following SOPs were distributed September 1, 2005:

- MDP-MTH-07, Detection of Pathogenic *E. coli* in Fresh Produce by Multiplex PCR (Revision 01)
  (Attachment 1, mPCR Validation Protocol has been archived)
- MDP-QA-03, Quality Assurance (QA) Controls, (Revision 03)
  Attachment 1, Current QA Control Strain Information
  Attachment 2, QC Control Failure Reporting Form

The following SOPs will be distributed December 1, 2005:

- MDP-LABOP-02, Sample Receipt, Elution, Preenrichment, and DNA Extraction (Revision 05)
  Attachment, Fabrication of the California Cantaloupe Shaker Adapter
- MDP-SHIP-03, Procedures for Packaging, Shipping, and Archiving Microbiological Cultures (Revision 02)
  Attachment 1, Shipment Destinations, Contacts, and Schedules
  Attachment 2, MDP Participating Laboratories Addresses and Contact Staff for Shipment of Cultures
  Attachment 3, Flowcharts of Shipments
  Attachment 4, MDP Shipment Form
- MDP-MTH-01, *Escherichia coli* MPN Method (Revision 05)
- MDP-MTH-03A, Isolation and Identification of *Salmonella* from Fresh Produce using Cultural Methods (Revision 01)
- MDP-MTH-04, Detection of *Salmonella* in Fresh Produce by BAX® PCR (Revision 02)
- MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX® PCR (Revision 02)
- MDP-MTH-06, *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Identification (Revision 02)
- MDP-MTH-07, Detection of Pathogenic *E. coli* in Fresh Produce by Multiplex PCR (mPCR) and Cultural Isolation and Identification (Revision 02)

D. Archiving and Additional Testing:

Archival of Isolates: NSL, Gastonia, North Carolina has been established as a centralized location for archival of isolates as well as a distribution center for isolates from MDP testing laboratories to the reference laboratories.

Additional Testing by Reference Laboratories: All target organisms are frozen in Microbank™ vials and shipped to NSL. Vials are shipped by NSL to the FDA/Center for Veterinary Medicine (CVM) laboratory in Laurel, Maryland for antimicrobial resistance testing and inclusion in the National Antimicrobial Resistance Monitoring System (NARMS) and pulsed-field gel electrophoresis (PFGE) analysis for inclusion in PulseNet. *Salmonella* and *E. coli* O157 isolates are also serotyped by FDA/CVM. Pathogenic *E. coli* isolates are shipped by NSL to Penn State for serotyping.

AMS will transfer data to Centers for Disease Control and Prevention (CDC) and FDA on a semi-annual basis.

E. Future Program Directions:

<u>*Shigella*</u>*:* Four *Shigella* species, serotypes A-D, (S. *dysenteriae, S. flexneri, S. boydii*, and *S. sonnei*, respectively) have been identified as human pathogens and are therefore of interest to the MDP program for screening purposes. MPO is collaborating with DCLS to develop a realtime PCR-based detection method for *Shigella* which will include enrichment using UPB and DNA clean-up, followed by cultural isolation and identification steps. Prior to program-wide introduction, all methods will be verified by the participating laboratories. Pending method development, MPO anticipates addition of *Shigella* as a target organism in calendar year 2006.

MDP Sample Shipping Assignment Chart

EFFECTIVE JANUARY 1 - MARCH 31, 2006 **1ST QUARTER 2006**

Deletions: Parsley/Cilantro (All States)

Additions: Alfalfa Sprouts (All States)

STATE COLLECTING SAMPLES (# OF SAMPLES)	Alfalfa Sprouts (SR)	Cantaloupe (CN)	Green Onions (GO)	Lettuce (LT)	Tomatoes (TO)
				Leaf/Romaine	
	3 Units	3 Units	3 Units	3 Units	3 Units
	10/05	07/02	07/04	04/01	05/01
CALIFORNIA - (14)	CA4	CA4	CA4	CA4	CA4
COLORADO - (2)	CO4	CO4	CO4	CO4	CO4
FLORIDA - (7)	FL4	FL4	FL4	FL4	FL4
MARYLAND - (4)	OH4	OH4	OH4	OH4	OH4
MICHIGAN - (6)	MI4	MI4	MI4	MI4	MI4
MINNESOTA - (2)	MN4	MN4	MN4	MN4	MN4
NEW YORK - (9)	NY4	NY4	NY4	NY4	NY4
OHIO - (6)	OH4	OH4	OH4	OH4	OH4
TEXAS - (8)	US4	US4	US4	US4	US4
WASHINGTON - (4)	WA4	WA4	WA4	WA4	WA4
WISCONSIN - (2)	WI4	WI4	WI4	WI4	WI4

LAB CODES:

California,McClellan - CA4
Colorado, Denver - CO4
Florida, Tallahassee - FL4
Michigan, East Lansing - MI4
Minnesota, St Paul - MN4
New York, Albany - NY4
North Carolina, Gastonia - US4
Ohio, Reynoldsburg - OH4
Washington, Olympia - WA4
Wisconsin, Madison - WI4

SAMPLE WEIGHT/SIZE: *(Gram amounts listed for Lab use only)*

* Lettuce - 7oz. (200 grams) Minimum
* Tomatoes - 4oz. (100 grams) or 3½ - 4" Minimum
* Alfalfa Sprouts - 4 oz (100 grams) Minimum
* Cantaloupe - 1.5lbs. (681 grams) Minimum
* Green Onions - 7 oz (200 grams) Minimum

MDP Sample Shipping Assignment Chart

EFFECTIVE APRIL 1 - JUNE 30, 2006 **2ND QUARTER 2006**

Deletions: NONE **Additions: NONE**

STATE COLLECTING SAMPLES (# OF SAMPLES)	Alfalfa Sprouts (SR)	Cantaloupe (CN)	Green Onions (GO)	Lettuce (LT)	Tomatoes (TO)
				Leaf/Romaine	
	3 Units	3 Units	3 Units	3 Units	3 Units
	10/05	07/02	07/04	04/01	05/01
CALIFORNIA - (14)	CA4	CA4	CA4	CA4	CA4
COLORADO - (2)	CO4	CO4	CO4	CO4	CO4
FLORIDA - (7)	FL4	FL4	FL4	FL4	FL4
MARYLAND - (4)	OH4	OH4	OH4	OH4	OH4
MICHIGAN - (6)	MI4	MI4	MI4	MI4	MI4
MINNESOTA - (2)	MN4	MN4	MN4	MN4	MN4
NEW YORK - (9)	NY4	NY4	NY4	NY4	NY4
OHIO - (6)	OH4	OH4	OH4	OH4	OH4
TEXAS - (8)	US4	US4	US4	US4	US4
WASHINGTON - (4)	WA4	WA4	WA4	WA4	WA4
WISCONSIN - (2)	WI4	WI4	WI4	WI4	WI4

LAB CODES:

California,McClellan - CA4
Colorado, Denver - CO4
Florida, Tallahassee - FL4
Michigan, East Lansing - MI4
Minnesota, St Paul - MN4
New York, Albany - NY4
North Carolina, Gastonia - US4
Ohio, Reynoldsburg - OH4
Washington, Olympia - WA4
Wisconsin, Madison - WI4

SAMPLE WEIGHT/SIZE: *(Gram amounts listed for Lab use only)*

* Lettuce - 7oz. (200 grams) Minimum
* Tomatoes - 4oz. (100 grams) or 3½ - 4" Minimum
* Alfalfa Sprouts - 4 oz (100 grams) Minimum
* Cantaloupe - 1.5lbs. (681 grams) Minimum
* Green Onions - 7 oz (200 grams) Minimum